## Differential Fluorimetric Estimation of Adrenalin and Noradrenalin

Fluorimetric methods for the differential estimation of adrenalin and noradrenalin, based on the oxidation of these amines to the corresponding 3,5,6-trihydroxyindoles, adrenolutine and noradrenolutine, differ from one another by the use of various oxidants, the pH at which oxidation is performed, and the selection of different excitation and fluorescence wavelengths[1-5].

In the more recent method of MERRILLS[6,7], as well as in its modification by ROBINSON and WATTS[8], the fluorescence due to both adrenalin and noradrenalin is measured in the presence of alkaline ascorbate, whereas thioglycollic acid is used as a stabilizer for the measurement of the fluorescence due to noradrenalin only. Indeed, the fluorescence due to adrenalin is immediately and almost completely suppressed when thioglycollic acid is used in place of ascorbic acid. However, a rapid decline also occurs for the noradrenalin fluorescence. In fact this method, which was developed as an autoanalytical procedure, requires very careful timing and is rather difficult to apply manually.

The method described here for the differential fluorimetric estimation of adrenalin and noradrenalin has been adapted mainly from the techniques of ANTON and SAYRE[5], MERRILLS[6,7], and ROBINSON and WATTS[8]. It has the advantage of simplicity and high sensitivity.

*Technique.* Reagents: (1) $K_3Fe(CN)_6$ 0.25% in water. (2) 0.5 *M* phosphate buffer, pH 7.0: bring a 1 *M* $KH_2PO_4$ solution to pH 7 by means of 1 *N* NaOH and dilute to 0.5 *M* with water. (3) Alkaline ascorbate: 10 mg ascorbic acid, 0.1 ml water, 5 ml 10 *N* NaOH; prepare fresh daily. (4) Cysteine-thioglycollic acid-ethanol mixture: 1% cysteine, 0.01% (v/v) thioglycollic acid (80%) and 5% (v/v) ethanol in 10 *N* NaOH; prepare fresh daily and centrifuge before use.

Procedure. (1) Estimation of adrenalin + noradrenalin: to 0.2 ml of catecholamine solution in 0.01 *N* HCl, add 0.017 ml of phosphate buffer, pH 7 (resulting in pH 6.8), and 0.02 ml of $K_3Fe(CN)_6$; after 1 min add 0.2 ml of alkaline ascorbate and 0.5 ml of water; after shaking, measure the relative fluorescence at excitation and fluorescence wavelengths of 409 nm and 519 nm respectively; a blank is obtained by omitting $K_3Fe(CN)_6$. (2) Estimation of noradrenalin only is done by using a cysteine-thioglycollic acid-ethanol mixture in place of alkaline ascorbate as stabilizer and measuring the fluorescence 15 min later.

The course of stabilization for both catecholamines (0.1 $\mu$g/ml 0.01 *N* HCl) in function of time shows (Figure 1) that in the presence of cysteine-thioglycollic acid the adrenalin fluorescence is reduced to zero within 15 min, whereas at that time the fluorescence due to noradrenalin can still be very adequately measured. The fluorescence for both adrenalin and noradrenalin in the 0.01–0.1 $\mu$g/ml range is illustrated in Figure 2.

Calculation. The absolute quantities of adrenalin and noradrenalin are calculated using the following formulas:

$$x_1 = \frac{a\,X_1}{X_2 - X_1} \quad \text{and} \quad x_2 = \frac{Y_1 - (Y_2 - Y_1/a)x_1}{Y_3 - Y_1/b}$$

where: $x_1$ = concentration of noradrenalin in $\mu$g/ml;
$x_2$ = concentration of adrenalin in $\mu$g/ml;
$a$ = quantity of noradrenalin used as internal standard;
$b$ = quantity of adrenalin used as internal standard;
$Y_1$ = fluorescence of adrenalin + noradrenalin in unknown sample;
$Y_2$ = fluorescence of unknown sample + internal noradrenalin standard;
$Y_3$ = fluorescence of unknown sample + internal adrenalin standard;
$X_1$ = fluorescence of unknown sample;
$X_2$ = fluorescence of unknown sample + internal noradrenalin standard.

*Results.* The results of estimations of 3 different proportions of noradrenalin (NA) and adrenalin (A) concentrations are given in the Table.

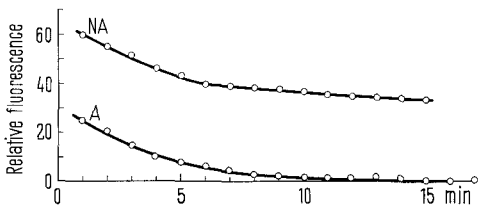


Fig. 1

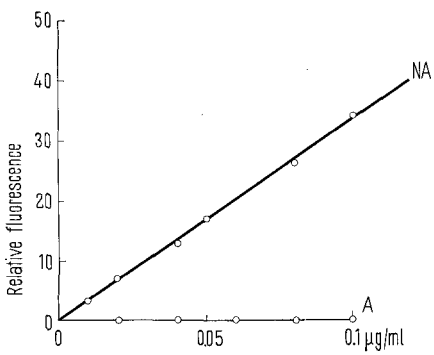


Fig. 2

| Concentration of CA in sample in $\mu$g/ml | $X_1$ | $X_2$ | $Y_1$ | $Y_2$ | $Y_3$ | $x_1$ | $x_2$ |
|---|---|---|---|---|---|---|---|
| NA 0.05<br>A 0.03 | 15 | 47 | 23 | 47 | 60 | 0.047 | 0.031 |
| NA 0.03<br>A 0.08 | 10 | 42 | 37 | 62 | 73.5 | 0.031 | 0.080 |
| NA 0.08<br>A 0.02 | 24 | 54 | 26 | 51 | 60 | 0.080 | 0.018 |


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*Zusammenfassung.* Einfache und empfindliche Methode zur differentiellen fluorimetrischen Bestimmung von 0.01–1 µg/ml Menge von Adrenalin und Noradrenalin. Die Oxydation wird mit $K_3Fe(CN)_6$ bei pH 6.8 durchgeführt. Nachdem die fluorescierenden Derivate von Adrenalin und Noradrenalin sowie von Noradrenalin allein mittels basischem Askorbat bzw. Cystein-Thioglykolsäure stabilisiert worden sind, wird die Fluorescenz bei 409 nm und 519 nm gemessen[9].

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## Measurements of the Flow of Aqueous Humor According to a New Principle

Though several methods have been devised for determining the flow of aqueous humor, none of them is sufficiently perfect to make a new approach to the problem uninteresting. The method to be described, which aims at a determination of the flow through the pupil, is based on the following observations.

If fluorescein in a solution is instilled into the conjunctival sack it penetrates the cornea and colours the aqueous humor. The penetration can be enhanced by iontophoresis, and in ca. 15 min the aqueous humor is strongly coloured. By movements of the eye the staining of the content of the anterior chamber can be made fairly homogeneous. The method relies on the fact that the newly-formed aqueous humor which emerges from the pupil is uncoloured and remains observable as a clear, slowly increasing volume, well demarcated against the green content of the anterior chamber for up to about 30 sec (Figure 1). After that time convection and diffusion make the boundaries of the volume indistinct and 'Schlieren' are formed. However, the content can be mixed again and the growing volume can be observed anew an arbitrary number of times. A flow value in absolute units might be obtained if the volume of the clear-growing 'vesicle' could be measured on 2 occasions at a known interval.

A method for estimating the volume of superficial tumours by means of 'Lichtebeneschnitte' has recently been described[1]. A number of parallel equidistant slits are projected over the surface of the tumour. This is photographed from an angle, fixed to the projector axis. On the photograph a series of lines are seen, deviating over the surface of the tumour. The areas between the deviations and the corresponding base lines are planimetered and the volume is easily estimated when the distance between the slit images is known. In trying to apply this new method to the present situation, it had to be modified by taking a series of single slit pictures in rapid succession (12/sec) with a cine-camera. The distance between the adjacent slit images was 0.28 mm (Figure 2).

The best measuring conditions are obtained with miotic pupils. The convection currents in the anterior chamber may disturb the formation of a well-defined 'vesicle' but it seems to be possible to master this difficulty by, for instance, irrigation of the cornea with warm or cold water. A number of experiments on rabbits have given flow values inside the normal range (1.5–3.8 $mm^3$/min). Al-

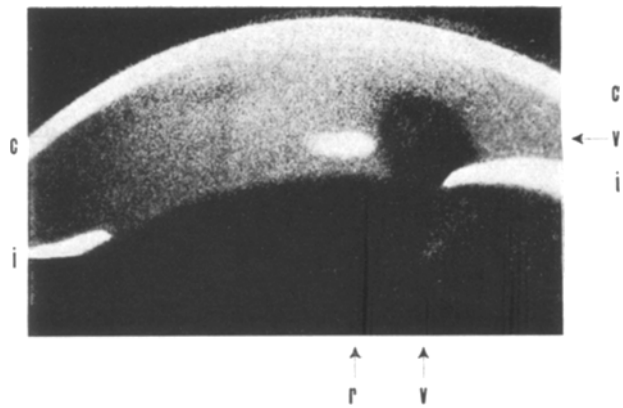


Fig. 1. Slit image giving an optical section through the auterior chamber; c = cornea, i = iris. The content is fluorescent apart from a clear 'vesicle' (v) at the pupillary border. Beneath the 'vesick' is the corneal reflex (r).

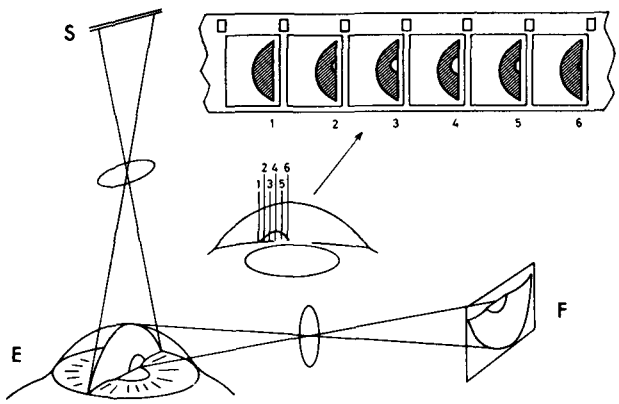


Fig. 2. Schematic diagram of measuring device. S = slit, illuminated from behind, which is projected on the eye (E) and photographed (F). The positions of the sections in a series of exposures and the corresponding images are shown.

[1] O. HOLM and C. E. T. KRAKAU, Acta universitatis lundensis, Sectio II 1965, No. 31.